Intramural papers of the month

By Kelly Lenox, Jacqueline Powell, Bailey Schug, and Deepa Singh

tssRNAs associated with paused Pol II serve as scaffold for transcription factors

Researchers from NIEHS have found that nascent transcription start site-associated (tss) RNAs, produced and stably bound by RNA polymerase II (Pol II) that has paused during early elongation, could provide a target for the recruitment of factors that modulate gene expression. Since transcription is a critical step in the creation of proteins from information within the genome, this work may provide insight into environmentally responsive gene expression and identify novel approaches for treating disease.

The scientists developed a highly sensitive method of characterizing the dynamics of promoter-associated Pol II and tssRNAs generated during early elongation in fruit fly, or *Drosophila*, cells. They performed a biochemical fractionation procedure that allowed separation of the short, capped RNA species, and identified their origins genome-wide using high-throughput sequencing. They found that paused Pol II and associated tssRNAs were very stable, long-lived species, remaining near gene promoters for tens of minutes before resuming transcription elongation.

The researchers propose that tssRNAs provide a physical framework on which transcription factors that regulate productive elongation and promoter chromatin could bind to release paused Pol II into productive elongation, thereby modulating gene expression. **(KL)**

Citation: Henriques T, Gilchrist DA, Nechaev S, Bern M, Muse GW, Burkholder A, Fargo DC, Adelman K. *(http://www.ncbi.nlm.nih.gov/pubmed/24184211)*
2013. Stable pausing by RNA polymerase II provides an opportunity to target and integrate regulatory signals. Mol Cell 52(4):517-528.

Asthmatic reaction is dependent on dose and timing of endotoxins

NIEHS scientists showed that immune responses to inhaled allergens are dependent on quantity and time of exposure to endotoxins. Two different arms of the adaptive immune response contribute to allergic asthma - T helper (Th) 2 cells and Th17 cells. These two arms of the immune response, which respond differently to factors in the environment, account for some of the heterogeneity seen in asthma. This knowledge offers potential opportunities for therapies that disrupt specific pathways associated with one or the other of these types of asthma.

Allergic sensitization, which is the biological basis for allergic asthma, is caused by allergens found in plants, insects, and animals. However, it is also caused by adjuvants, or ingredients that increase the immune response, such as those found in microbial products, including lipopolysaccharide (LPS). The researchers used a mouse model to demonstrate that immune responses to inhaled allergens are highly dependent on the doses of inhaled LPS, including the amounts found naturally in the environment. They discovered that low doses of LPS promote classical, Th2-driven allergic responses to inhaled allergens, whereas moderate doses of endotoxin induce stronger Th17 responses and associated neutrophilia.

The researchers also demonstrate that inhalation of moderate doses of LPS during sensitization induces regulatory responses that, after multiple allergen exposures, limit the severity and longevity of asthma-like features. **(BS)**

Citation: Whitehead GS, Thomas SY, Cook DN. *(http://www.ncbi.nlm.nih.gov/pubmed/24168764)*
2013. Modulation of distinct asthmatic phenotypes in mice by dose-dependent inhalation of microbial products. Environ Health Perspect; doi:10.1289/ehp.1307280 [Online 29 October 2013].

Modest changes in dNTP levels affect cell's ability to repair mutations

Recent studies by NIEHS researchers have indicated that the cellular levels of deoxynucleoside-5'-triphosphates (dNTPs), the building blocks for DNA, have to be strictly regulated to maintain low mutation rates. The main enzyme responsible for

controlling dNTPs is ribonucleotide reductase (RNR) and mutations in RNR have a direct bearing on the rate of DNA mutations.

The researchers isolated *Escherichia coli* mutant RNRs and found two new alleles in which dGTP and dATP concentrations were affected almost twofold, up versus down, respectively. Interestingly, despite these modest dNTP changes, the mutation rate in the *rpoB* gene encoding the beta subunit of bacterial RNA polymerase was increased more than 3,000-fold. They hypothesized the change could be due, in part, to collapse of the mismatch repair (MMR) system that normally removes most errors post-DNA replication.

The scientists used several approaches to explain the high mutability of these two RNR mutants. They measured mutation frequencies in MMR-proficient and deficient backgrounds, and observed that the MMR is no longer active in these two mutants. Similarly, overproduction of one of the components of the MMR system (*mutL*) or the inclusion of a *dnaQ926* antimutator allele lowered the mutation frequencies, indicating the restoration of an active MMR system.

Overall, these studies indicate that abnormal levels of dNTPs can saturate MMR and can lead to hypermutability and error catastrophe. **(DS)**

Citation: Ahluwalia D, Schaaper RM.
(http://www.ncbi.nlm.nih.gov/pubmed/24167285)
2013. Hypermutability and error catastrophe due to defects in ribonucleotide reductase. Proc Natl Acad Sci U S A 110(46):18596-18601.

Distinct features of RNA binding protein make it unique among its family

NIEHS scientists and their collaborators recently uncovered unique structural features of the tandem zinc finger (TZF) domain of tristetraprolin (TTP), an RNA-binding protein. TTP is the best-known member of the TTP family of tandem zinc finger proteins that promote the degradation of target mRNAs.

While TTP family proteins have highly conserved TZF domains that are critical for RNA binding, researchers performed a cross-species TZF domain sequence alignment and uncovered seven residues that were highly specific to TTP. By generating a structural model of TTP based on the known structure of another TTP family protein, ZFP36L2, they discovered that the second zinc finger of TTP was structurally distinct from the first zinc finger of TTP and both zinc fingers of ZFP36L2. These differences were mainly observed in the C-x8-C intervals, where six of the seven TTP-specific residues were located. Researchers then mutated residues within the TZF domain of TTP to examine their role in RNA binding. Mutating sequence equivalent residues in the first and second zinc fingers of TTP often had different effects on RNA binding, indicating that the two zinc fingers of TTP contain functionally distinct residues.

This research suggests that although the TZF domain of TTP family proteins is highly conserved, they may bind to RNA differently, based on subtle structural differences. **(JP)**

Citation: Lai WS, Perera L, Hicks SN, Blackshear PJ.
(http://www.ncbi.nlm.nih.gov/pubmed/24253039)
2013. Mutational and structural analysis of the tandem zinc finger domain of tristetraprolin. J Biol Chem; doi:10.1074/jbc.M113.466326 [Online 19 November 2013].

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